EFFECTS OF AERATION, LIGHT AND TEMPERATURE ON SPORE GERMINATION AND GERM TUBE GROWTH OF *CURVULARIA PALLESCENS* BOED.

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ABSTRACT - Spore germination and germ tube growth of *Curvularia pallescens* were studied under various light and dark regimes, by observing spores in suspension drops on glass slides without coverslips, with normal coverslips, or with shimmed coverslips. These studies were carried out at temperatures: 10, 15, 20, 25 and 30°C. Light (1,133 lux) good aeration and temperatures of 25-30°C significantly promoted spore germination and intercalary outgrowth. By contrast post germination elongative growth was also promoted regardless of light or dark conditions.

RÉSUMÉ - La germination des spores et la croissance du tube germinatif de *Curvularia pallescens* ont été étudiées en régimes lumineux ou obscurs en comparant à des températures échelonnées entre 10 et 30°C leur développement en suspension dans des gouttes dépourvues ou couvertes de lamelles appliquées telles quelles ou surélevées par des débris de celles-ci. La lumière (1.133 lux), une bonne aération et des températures de 25-30°C ont stimulé de manière significative tant la germination que l'émergence intercalaire de tubes. Par contraste, l'élongation post-germinative des tubes a été aussi stimulée mais indépendamment de l'alternative lumière-obscurité.

KEY WORDS : *Curvularia pallescens*, spore germination.

INTRODUCTION

The ability of plant pathogenic fungi to infect their hosts depends upon the germinability of their spores and the vigour of germ tube growth. Environmental conditions may alter the ability of fungi to produce germ tubes and infection structures.

The spores of some fungi do germinate and produce appressoria under low oxygen tensions or high concentrations of gases such as CO_2 (Buston et al., 1966; Schanel, 1976). Oxygen tension and CO_2 concentration during spore germination could be modified by different methods of coverslip placement on a spore suspension or by not placing a coverslip (Russo et al., 1979). Light is necessary for spore germination and germ tube growth of some fungi (Cole & Geeligs, 1977; Khan, 1977). Laboratory techniques could be used to test the effect of light quantity and quality on spore germination (Cochrane, 1958; Ekpo & Esuruoso, 1977; Lapp & Skoropad, 1976).

Curvularia pallescens Boed. with multi-celled conidia, has the capability of producing more than one germ tube, and this attribute is believed to contribute to the success of *C. pallescens* as a pathogenic fungus (Mabadaje, 1969).

The purpose of this study was to determine effects of aeration, light and temperature on spore germination and germ tube growth of *C. pallescens*. This study was motivated by the idea that the information obtained might provide a basis for manipulation of the fungus in future physiological studies involving growth and infection processes.

MATERIALS AND METHODS

The isolate of *C. pallescens* used during the study was obtained from infected maize leaves at the experimental plot of the National Cereals Research Institute, More Plantation, Ibadan, Nigeria.

Spore suspensions were prepared by flooding with sterile deionized water, 12 day-old sporulating cultures of *C. pallescens* and dislodging spores with a bent glass rod. The suspensions were filtered through a double layer of sterile muslin cloth, to remove the hyphae. The filtered suspensions were poured into McCartney bottles and centrifuged at 2,750 g for 5 mins. After washing three times, the spore suspensions were standardised to 1.0×10^4 spores per ml of deionized water with the aid of a Fusch's Rosenthal haemocytometer counting apparatus supplied by NAAFCO Scientific Supplies Nig. Limited (Lagos, Nigeria). Spore suspension drops of about 0.1ml were placed on glass microscope slides above moistened filter paper in petri-dishes. The suspension was left uncovered (UC) or covered with a coverslip (CC) or with shimmed coverslips (SC) and incubated at temperatures of 10, 15, 20, 25 and 30°C up to 24 hours, under continuous cool-white fluorescent light (1,133 lux) or in complete darkness. The experiment was replicated five times. Shims were made by placing intact coverslips on droplets of spore suspension surrounded by pieces of broken coverslips. The procedure increased aeration and gas exchange in the spore suspension and was similar to the method used by Russo et al. (1979). Gallenkamp incubators with and without illumination were used for controlling the various temperature regimes and light conditions.

Observations were made at 6, 12, 18 and 24 hours after incubation similar to the method described by Russo et al. (1979). Three microscope fields each were examined along three axes: 0.3mm (LI), 4-7mm (L2), and 8-11mm (L3) from the approximate centre of coverslips for CC and SC. A different method was applied to the UC since the droplets did not disperse until the coverslips were placed on them before viewing. Coverslips were carefully applied onto the suspension, to prevent extensive dispersal of spores. Observations were made in the same manner as for the CC and SC treatments. Percentage spore germination, germination in more than one cell of the spore (intercalary germination), number of germ tubes and average length of main tubes per spore were recorded.

RESULTS

The results were similar for all incubation periods, and therefore only results obtained at 24 hr after incubation are presented. The effects of light and gaseous exchange on *C. pallescens* were significant at all incubation temperatures. However, temperature effect was major and significant up to 25°C, after which there was no significant effect due to further increase in temperature (Fig. 1). Spores incubated under light germinated better than those in the dark (Fig. 1). Furthermore, SC under light incubation significantly promoted spore germination more than SC under dark as well

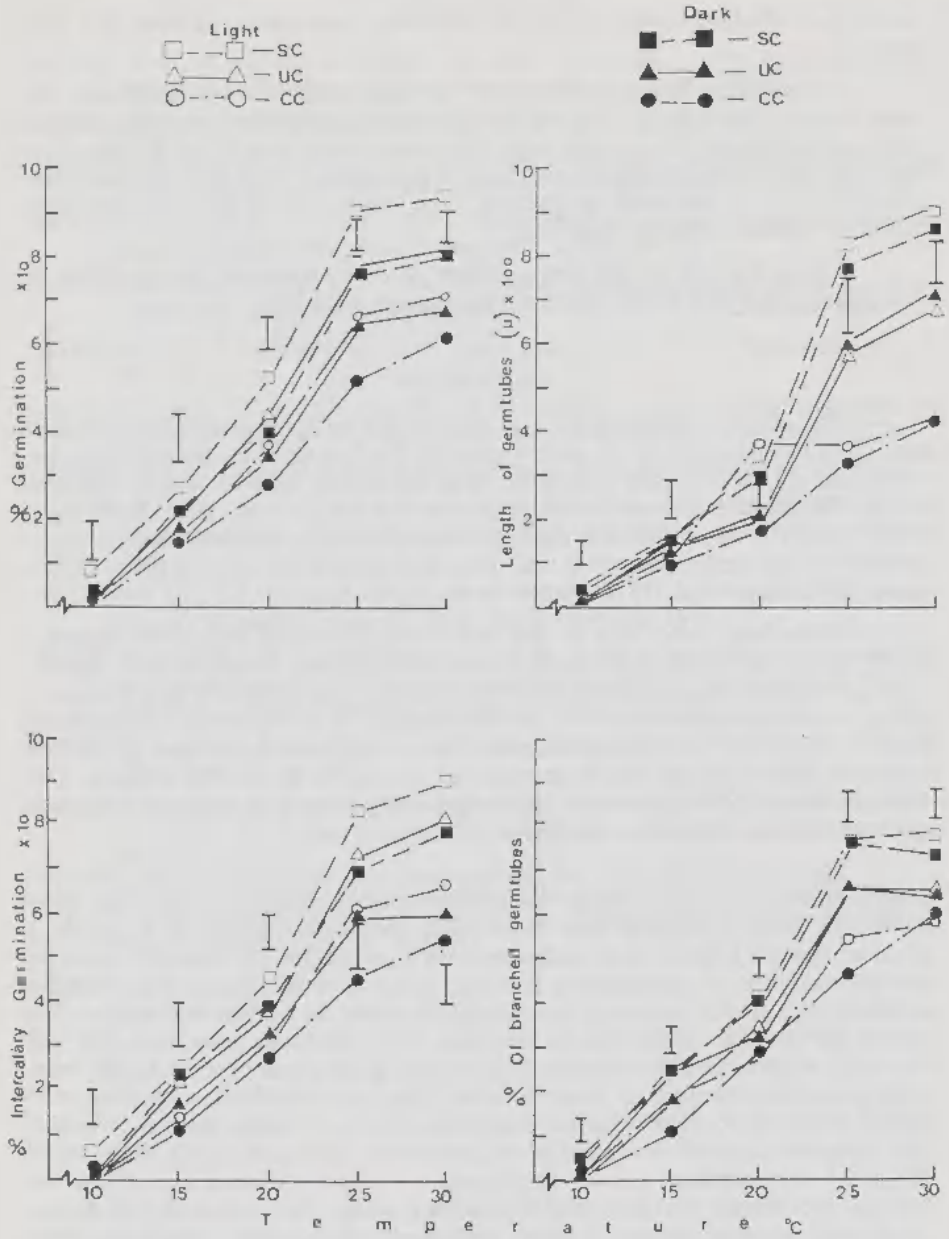

Fig. 1. The effect of light, gaseous exchange and temperature regimes on germination and germ tube growth of *Curvularia pallescens* spores after 24 hours of incubation in droplets without coverslips (UC), normal coverslips (CC) and shimmed coverslips (SC).

Fig. 1 - Effet de la lumière, des échanges gazeux et de la température sur la germination des spores et la croissance du tube germinatif de *Curvularia pallescens* après 24h d'incubation dans des gouttes non couvertes de lamelles (UC), couvertes de lamelles (CC) ou lamelles surélevées (SC).

as light and conditions with UC or CC. Intercalary germination followed this same trend.

Elongation as well as branching of the germ tubes were not significantly different under either light or dark regimes, but spore suspension in the SC treatment produced significantly longer germ tubes than those formed with UC or CC. In all the light and temperature treatments, branching and elongation were least without coverslips (UC) (Fig. 1), but here, spores lying close to the edge of the suspension drops did differ markedly from the inner ones.

Due to the fact that the fungus did not produce appressoria throughout the investigations, no record of this structure was included in the data.

DISCUSSION

The effects of temperature, light and aeration on spore germination and germ tube growth of fungi can not be under-estimated. The impact of temperature on germination and growth of several pathogenic fungi has already been stressed by Cochrane (1958). The effect of gaseous exchange (Medeiros & De Ti Alvin, 1967; Russo et al., 1979; Wallace et al., 1976), and the importance of light or dark incubation on spore germination and germ tube growth and development has been reported (Ekpo & Esuruoso, 1977; Russo et al., 1979; Wallace et al., 1976).

Some fungi prefer light to dark period for germination and growth (Russo et al., 1979) and the results of this study indicate that light may be one activator agent of spore germination in *C. pallescens*. However, light or dark apparently have little or no effect on post germination growth and development of *C. pallescens*. Temperature played a major role in fungal germination and the optimum temperature of 25-30°C needed no doubt favoured enzymatic reactions involved in germination processes (Jefferies & Young, 1976). Intercalary germination was promoted by the same light, gaseous exchange and temperature conditions.

Spore germination with the SC treatment was significantly higher than with other treatments. Gaseous exchange can promote spore germination (Toler et al., 1966) while poor gaseous exchange may inhibit spore germination (Macko et al., 1976). It could be that since spores of *C. pallescens* sank in water, the UC treatment could not provide conditions of good gaseous exchange in the spore suspension. The placing of shimmed (SC) as well as normal coverslips (CC) breaks the surface tension of the water and allows more aeration than no coverslips (UC). Although germ tubes of *C. pallescens* developed equally well under light or dark, growth was better in the SC treatment presumably because of better aeration. This result confirms a previous study (Russo et al., 1979). However, temperature was the major factor since it influenced post germination growth as well as spore germination (Cochrane, 1958). Thus, for infection of the host, *C. pallescens* would need optimum environmental conditions before leaf spot disease of maize could maximally develop. Germination of *C. pallescens* spores may be aided by light, gaseous exchanged and optimum temperatures, while germ tube growth and development may be promoted by aerobic conditions at an optimum temperature in either the light or the dark. The quantity and quality of gaseous exchange and light are areas that need further investigation.

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